Peer Review
Experimental Toxicology Division
N H E E R L

PARTICULATE MATTER

Characteristics of Particulate Matter Which Enhance Respiratory Tract Responses in a Mouse Model of Allergic Asthma

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Environmental Issue and Objectives

- Respiratory morbidity and mortality associated with increases in ambient levels of particulate matter (PM) may be dependent on particle elemental composition.
- Particle-associated metals such as copper may catalyze formation of reactive oxygen species leading to inflammation and lung injury.
- We have studied the ability of ambient air particles and chemically defined synthetic particles to enhance allergic inflammation and airway hyperresponsiveness in ovalbumin (OVA)-allergic mice.
- The goal of these studies is to describe the relative importance of PM components in the enhancement of allergic airways responses and contribute to the understanding of mechanisms of susceptibility in sensitive populations.

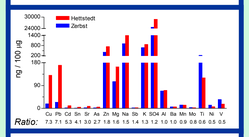
Linkage of Epidemiology with Toxicological Effects of Ambient $PM_{2.5}$

Background and Approach

- Children living in polluted Hettstedt, eastern Germany, in the early 1990's had higher lifetime prevalence rates of bronchitis and allergic diseases compared with children living in the nearby town of Zerbst which is relatively clean (Heinrich, *Env. Health Perspect.* 107:53-62, 1999).
- In order to link epidemiological results with allergic airways responses to specific components of air pollution, we analyzed elemental composition of $PM_{2.5}$ from Hettstedt and Zerbst collected in 1999, and exposed mice to $PM_{2.5}$ samples from these cities (100 µg total dose) immediately before the sensitization phase or the challenge phase of the allergic response to ovalbumin (OVA) antigen.

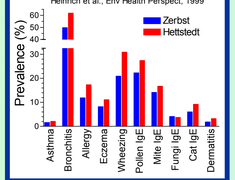
Piles of slag (smelted metal ore) in Hettstedt, where metals have been mined and processed for 800 years.

$PM_{2.5}$ Metal Content: Hettstedt > Zerbst

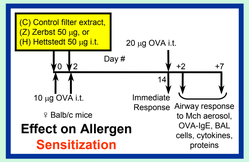


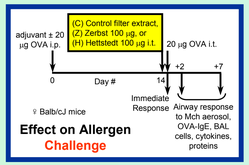
ICP Analysis: Aqueous Extracts of $PM_{2.5}$ Filters Collected January – June, 1999

Health Endpoints (1993)
Heinrich et al., Env Health Perspect, 1999



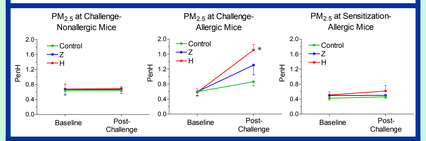
- Cross-sectional epidemiological study of children (5-14 yr old)
- Hettstedt children vs. Zerbst children:
  - ~ 2x level respiratory symptoms (wheeze, shortness of breath, cough without cold)
  - ~ 50% increased prevalence of allergies, eczema, bronchitis
  - Significantly increased sensitization to common aeroallergens





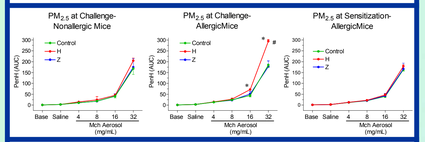
Buxco Measures of Airflow Obstruction

Immediate Responses to OVA Allergen Challenge



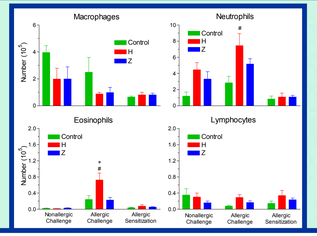
* Mice exposed to Hettstedt $PM_{2.5}$ 2 hr before OVA challenge (but not sensitization) were significantly more responsive than nonallergic mice instilled with Hettstedt $PM_{2.5}$.

Responses to Methacholine 2 Days after OVA Challenge



Mice exposed to Hettstedt $PM_{2.5}$ 2 hr before OVA challenge were hyperresponsive to Mch compared to *nonallergic Hettstedt-exposed mice and #allergic Zerbst $PM_{2.5}$ or control mice.

BAL Cells 2 Days after Challenge



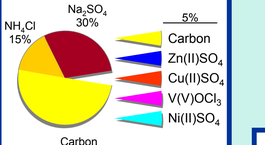
- Hettstedt $PM_{2.5}$ 2 hr before challenge caused increases in neutrophils and eosinophils vs. *nonallergic Hettstedt-exposed mice and #allergic Zerbst $PM_{2.5}$ or control mice.
- Only Hettstedt $PM_{2.5}$ caused significant increases in OVA-specific IgE, whether administered before sensitization or before challenge.
- Both Hettstedt and Zerbst $PM_{2.5}$ administered 2 hr before challenge caused significant increases in BAL protein, LDH, TNF-α and IFN-γ.

Synthetic Particles to Define the Role of Metals in PM-Induced Allergic Responses

Background and Approach

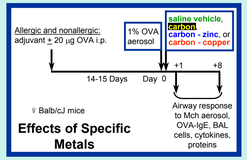
- Analyzing the effects of specific components of ambient PM in susceptible populations is an exceedingly complex problem. Use of synthetic particles is one approach to characterize the contribution of individual components.
- Particles (< 2 µm) were made which were composed of carbon, NH_4Cl, and Na_2SO_4. Control carbon (C) particles contained an extra 5% carbon, while metal-loaded particles contained 5% metal compound (2% pure metal).
- Pulmonary function and inflammation were assessed in OVA-allergic and nonallergic Balb/cJ mice after intratracheal instillation of synthetic particles (2 mg/kg, ~ 40 µg).

Synthetic Particle Composition

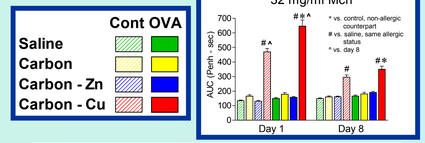


~ 0.33 mmol metal/gm PM
~ 2% pure metal by weight

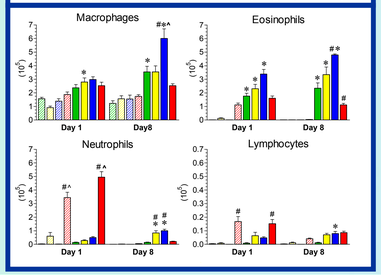
Chemicals were mixed in water/Triton X-100, aerosolized, dried at 17 lpm in a 60°C heated tube, and collected in a Teflon/fabric bag (Particle Technology, College Park, MD).



Cu-Containing Particles Cause Airway Hyperresponsiveness



Cu-containing Particles: Increased Neutrophils, Lymphocytes;
Zn-containing Particles: Increased Macrophages, Eosinophils



Results Summary: Cu-Containing Particles Exacerbate Allergic, Airway Responses

	Saline	Carbon	C / V^V	C / Ni^{II}	C / Zn^{II}	C / Cu^{II}
AHR - Mch	-	-	-	-	-	++++
Macrophages	-	+	-	-	++	-
Eosinophils	++	++	+	+	+++	+
PMNs	-	+	-	-	+	++++
Lymphocytes	-	+	-	-	+	++
BAL Injury	-	+	-	-	+	++
Th2 cytokines	+	+	nd	nd	+	++++
Pro-infl. cytokines	-	-	nd	nd	-	++++

Conclusions

Ambient $PM_{2.5}$

- Despite the closure of industries in Hettstedt since the reunification of Germany and lessening of differences in overall ambient PM levels, differences in elemental composition of $PM_{2.5}$ still persist, which may be attributable to metal mining and smelting industries in Hettstedt. Levels of copper, lead, and other metals were several-fold higher in Hettstedt $PM_{2.5}$ compared with Zerbst $PM_{2.5}$.
- Ambient $PM_{2.5}$ with high levels of metals caused increases in lung inflammation and airway hyperresponsiveness (AHR) in mice with allergic airways disease, but only when administered in previously sensitized mice immediately before allergen challenge. Allergic sensitization (OVA-IgE) was increased with either protocol.
- These results are consistent with previous epidemiological findings and further implicate metal composition of ambient PM as a component in the exacerbation, and possibly the development, of allergic respiratory diseases such as asthma.

Synthetic Metal Particles

- Synthetic particles containing Ni and V did not enhance allergic inflammation and AHR in mice. Particles containing Zn increased BAL macrophages and neutrophils but few other parameters.
- Synthetic particles containing copper caused significant increases in allergic inflammation, cytokines, and AHR.
- These studies point to copper as a likely component in PM-associated effects on allergic respiratory disease. They are consistent with recent studies relating toxicological effects (Dye, Env Health Perspect 2001; Ghio, AJRCCM 2001) with epidemiological findings of health effects of PM (Pope, Am J Public Health 1989). The ambient PM examined in these studies contained high concentrations of copper.

Impact / Future Directions

- The combination of epidemiological and toxicological approaches used here gives greater certainty in the understanding of health effects of ambient air $PM_{2.5}$. They suggest that metals associated with $PM_{2.5}$ affect the severity of pre-existing asthma (Gavett et al., *Env. Health Perspect.*, 111:1471-1477, 2003)
- Synthetic particles with precisely defined concentrations of metals will help us to understand which ambient air PM-associated metals may affect asthma and other cardiopulmonary diseases.
- The approaches used in these studies will be applied to examine responses following exposures to samples of source-category PM (oil, diesel, coal, or crustal source PM).
- These studies will help us to understand the mechanisms by which components of ambient air PM affects asthma and other cardiopulmonary diseases.

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